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Long-Term Survival and DNA Ploidy in Advanced Epithelial Ovarian Cancer

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Background and Objectives: The relationship[1] of the tumor DNA content to survival of patients with advanced epithelial cancer has not yet been clarified. A large amount of contradictory data exists in the literature. This study analyzes the putative relationship between ploidy and advanced ovarian carcinoma.

Methods: A retrospective analysis of tumor ploidy, DNA index, and the S-phase fraction from 35 patients with nonborderline epithelial ovarian carcinomas was determined by flow cytometry of paraffin-embedded tissue. All patients had FIGO stage III or IV disease. Those patients who survived >5 years were assigned to Group A (10 patients). Group B consisted of 25 age-matched subjects who succumbed to their disease within 5 years of diagnosis.

Results: Group A had not reached a median overall survival with a median follow-up of 114 months (range 67–226), whereas Group B had a median overall survival of 17 months (range 1–48). Two of the patients in Group A and all of the patients in group B had died of the disease. The two groups were similar in age, histologic type, and treatment. In Group A, three patients had grade 1 tumors, in contrast to group B where all the patients had either grade 2 or 3 disease ($P = 0.018$). However, the distribution of aneuploidy was similar in both groups. Also, the DNA indices were similar: 1.40 ± 0.42 in Group A, and 1.36 ± 0.44 in Group B. The median S-phase fraction was 14% (range 3–23%) in Group A, and 15% (range 2–23%) in Group B. The grade and type of tumor were not related to the ploidy or the DNA index. There was no significant correlation between ploidy or the DNA index and survival.

Conclusion: This study suggests that the DNA content of tumor as measured by flow cytometry is not a predictor of long-term survival in ovarian cancer patients with advanced disease.

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KEY WORDS: **ovarian cancer; flow cytometry; ploidy; long-term survival; cancer stage**

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INTRODUCTION

The majority of women with epithelial ovarian cancer have advanced disease at presentation. This observation translates into a 5-year overall survival rate of only ~20%, with a disease-free survival (DFI) of <10% [1]. Nonetheless, a fraction of these patients with very advanced disease has a better outcome when followed continuously over time.

In multivariate analysis of 726 patients with the International Federation of Gynecology and Obstetrics (FIGO) stages III-IV, the Gynecologic Oncology Group (GOG) showed correlation of age, performance status, stage, tumor volume, tumor type, and cisplatin chemotherapy with the overall survival [1]. For the last 15 years, flow cytometric analysis has been used as well. Notwithstanding a large body of research in this area, controversy still exists regarding prognostic significance of the tumor DNA content in advanced-stage cases of ovarian cancer. This study examines the putative relationship between ploidy and survival in advanced ovarian carcinoma in a group of patients with long-term follow-up.

MATERIALS AND METHODS

The Lutheran General Hospital Tumor Registry files were computer searched to identify 287 cases of ovarian cancer for the years 1976–1988. Subjects with FIGO stages III and IV ovarian cancer were eligible for inclusion into the study. The criteria for exclusion were: (1) nonepithelial histology, (2) tumors of low malignant potential, (3) patients whose primary staging/cytoreductive surgery was done at outside institutions, and (4) patients whose paraffin-embedded blocks were unavailable for analysis. Long-term survivors were defined as those patients still alive at 5 years following initial diagnosis. Ten patients fitting that description were found and assigned to Group A. Group B included 25 age-matched subjects who succumbed to their disease within 5 years of the diagnosis.

The patients hospital charts were reviewed and abstracted to record the demographic data, date of initial diagnosis, amount of residual disease at the end of primary surgery, subsequent therapy regimens, sites of recurrence, and date and status at last follow-up. The microscopic histologic material was reviewed by two pathologists who were blinded as to patient outcome. Tumor type was assigned according to previously published criteria [2]. Tumor was recorded as grade 1 if there was less than 5% solid growth pattern, grade 2 if 5–50% solid growth pattern, and grade 3 if greater than 50% solid growth pattern. Presence of significant nuclear atypia raised the grade of tumor by one level.

After examination of microscopic slides, areas of viable tumor were selected for analysis and the corresponding regions were marked on the paraffin blocks. Conventional flow cytometric technique [3] was used to determine tumor ploidy, DNA index (DI), and S-phase fraction (SPF). Five to 10 sections of 50 μm thickness were cut from the isolated tumor area. Disaggregation of the cells was accomplished by processing the sections in water, then pepsin, and finally trypsin. RNA interference was eliminated by the application of RNAse to the solution, according to the standard protocol [4]. The single-cell suspension was stained with propidium iodine and examined for nuclear DNA content by flow cytometry. The 20,000 cell data were collected and analyzed for each specimen. Standard DNA histograms were generated and were evaluable in all cases. The DI for each case was calculated by dividing the channel number of the aneuploid G_0/G_1 peak by the channel number of the diploid G_0/G_1 peak. Tumors were coded as diploid if the DNA index was <1.3. There were no purely tetraploid tumors.

Survival was plotted using the Kaplan-Meier method [5]. The Fisher exact test was used to compare the two groups as to demographic and microscopic data. Rank correlation was used to assess relationship between histopathologic, clinical, and flow cytometric characteristics. Chi-square analysis was performed to determine the association of DI, SPF, and ploidy with survival.

RESULTS

The median age of patients in Group A was 57.5 years (range 47–74), and the age-matched Group B had the median age of 57.0 years (range 34–74). In Group A, after the median follow-up of 114 months (range 67–226), two patients died of disease (DOD) at 67 and 85 months, three patients are alive with disease (AWD), and five patients are alive with no evidence of disease (NED). The median overall survival in Group A had not been reached (Fig. 1), whereas the median progression-free interval (PFI) was 72 months (Fig. 2). In Group B, where all of the patients were DOD, the median overall survival was 17 months (range 1–48) and the median PFI was nil.

All the patients in both groups received platinum-based combination therapy following the initial staging or cytoreductive surgery. Information on the amount of residual disease and performance status was insufficient for analysis.

Seven patients in Group A had endometrioid type tumors and three patients had serous type tumors (Table I). There were 11 serous, 11 endometrioid, one mucinous, one clear cell, and one undifferentiated tumor in Group B. In Group A, there were three patients with grade 1 tumors, in contrast to Group B where all the patients had either grade 2 or 3 disease ($P = 0.018$).

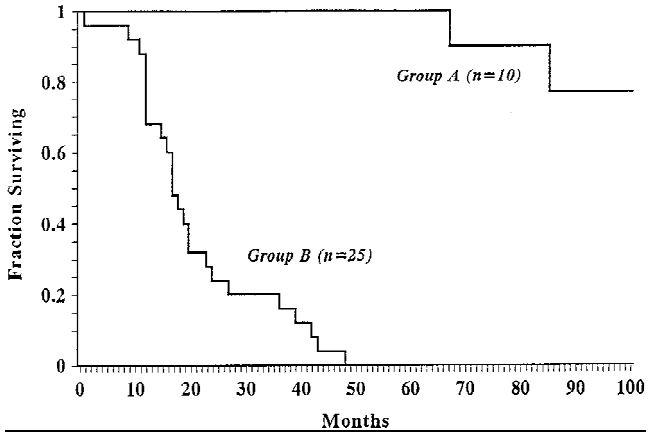

Fig. 1. Overall survival by groups. Whereas the patients in Group A have not reached the median survival after a median follow-up of 114 months, all of the patients in Group B were dead of the disease by 48 months of follow-up. Groups A and B were comprised exclusively of patients with Stage III and IV disease. The only statistically significant difference ($P = 0.018$) between the groups was tumor grade (See Table I).

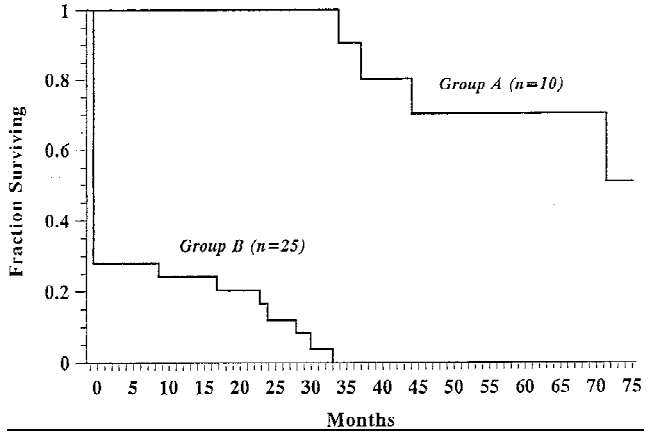

Fig. 2. Progression-free survival by groups. In Group A, the median progression-free interval (PFI) was 72 months; in Group B, the PFI was 0. Besides tumor grade, there were no other statistically significant differences between the groups.

DNA histograms were obtained in all of the 35 cases. The distribution of aneuploidy was similar in both groups (Table I). Figure 3 illustrates a representative case of a diploid tumor and Figure 4 illustrates a case of an aneuploid tumor. DNA indices and S-phase fractions were calculated from the histograms. DNA indices were similar in both groups: 1.40 ± 0.42 in Group A and 1.36 ± 0.44 in Group B. The median S-phase fraction was 14% (range 3–23%) in Group A, and 15% (range 2–23%) in Group B. The grade and type of tumor were not related to the ploidy or the DI. There was no statistically significant correlation between ploidy, SPF, or DI and survival.

TABLE I. Epithelial Ovarian Cancer: Clinical and Pathological Characteristics of the Study Patients

	Group A n = 10	Group B n = 25
Age		
median	57.5	57
range	(47–74)	(34–74)
Histology Type		
serous	3	11
endometrioid	7	11
mucinous	0	1
clear cell	0	1
undifferentiated	0	1
Grade		
1	3	0
2	4	8
	($P = 0.018$)	
3	3	17
Ploidy		
diploid	5	13
aneuploid	5	12
DNA Index	1.40 ± 0.42	1.36 ± 0.44
S-Phase Fraction		
median	14%	15%
range	(3–23)	(2–23)

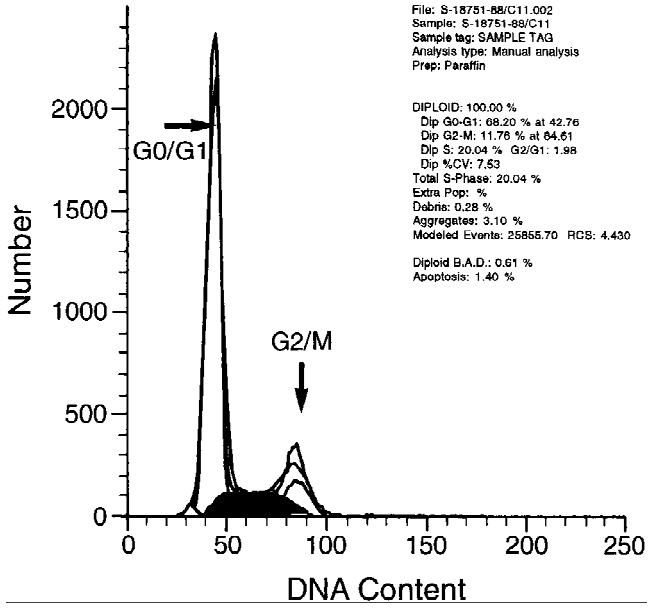

Fig. 3. Diploid histogram. Note a single G_0/G_1 peak. S-phase is shown as blackened area between G_0/G_1 and G_2/M peaks. It was calculated at 20% of the cell population.

DISCUSSION

Flow cytometry has been applied to the analysis of solid tumors for the past 15 years. Currently this technology is in widespread use. Since the reproducibility of other prognostic factors such as tumor grading and amount of the residual disease has been questioned, the hope has been that objective analysis of the DNA content of the tumor cells would be more valuable.

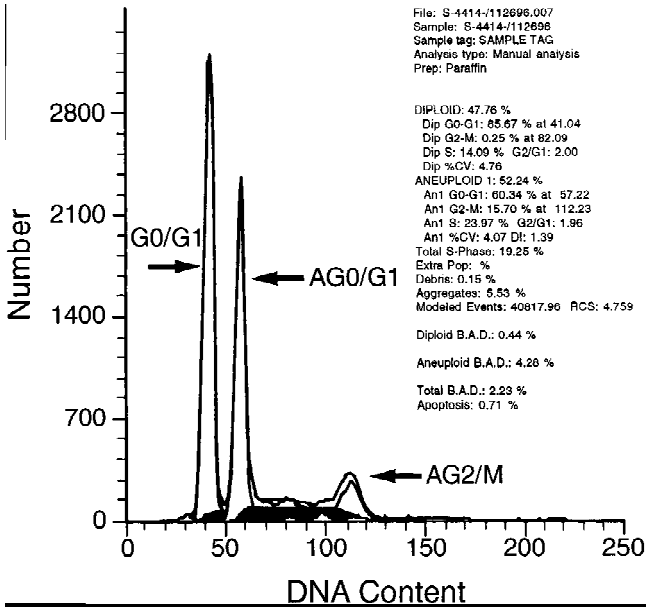

Fig. 4. Aneuploid histogram. Note two separate G_0/G_1 peaks (G_0/G_1 and AG_0/G_1). S-phase is shown as blackened area between AG_0/G_1 and AG_2/M peaks. It was calculated at 24% of the cell population.

A series of reports suggested that flow cytometric analysis of ovarian tumor DNA content was an objective, reproducible, and independent prognostic survival factor. In a frequently quoted study by Rodenburg et al. [6], ploidy was felt to be a major prognostic factor for survival in advanced ovarian cancer. This conclusion was applicable only to the 15 patients with ascites and small residual disease (<1.5 cm in diameter), where the median survival in six patients with diploid tumors was >60 months, whereas the median survival time of 22.6 months was observed in nine patients with nondiploid tumors ($P = 0.025$). Tumor ploidy was not a significant additional value in any other group.

However, subsequent larger studies have shown only a small incremental survival benefit to patients with diploid tumors. A study from Finland reported on 68 patients with FIGO Stages III and IV disease [7]. For these patients the median survival of those with diploid tumors was ~24 months as compared to those with aneuploid and multiploid tumors whose median survival was 12–14 months. In a study from Norway, the median survival of patients with diploid tumors was 18 months as compared to 8 months for those with aneuploid tumors [8]. A large study by the Australian Gynecologic Oncology [9] group reported on 128 ovarian cancer patients with Stages III and IV disease. The median survival was 13 months for patients with aneuploid tumors and 16 months for patients with diploid tumors.

The value of ploidy was questioned by some who found the S-phase fraction to be more predictive of survival [10]. It also has been suggested that DNA ploidy is associated with known predictors of survival, such as stage and grade [11,12], but that in stages III and IV there is no independent prognostic significance [13]. Most recently, several reports showed no statistically significant difference in overall survival between the patients with nondiploid and diploid tumors [12,14,15]. One of these reports [14] observed improved disease-free interval in patients with a DNA index <1.3; however, this was not corroborated in the other studies [12,15].

The results of the present study are particularly interesting considering that the patients in the long-term survival group had a remarkably long follow-up (median of 114 months, range 67–226). In addition, the influence of the age on the outcome was controlled by age-matching. Our retrospective data showed that the only significant difference between the long-term survivors of advanced ovarian cancer and their less fortunate counterparts was the grade of the tumor. Other parameters tested showed no statistical significance. The current study suggests that the significance of the DNA content of the cells as measured by flow cytometry in predicting the biologic behavior of advanced epithelial ovarian cancer is still unclear. The value of the DNA ploidy analysis in the care of patients with this disease appears to be limited at the present time.

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REFERENCES

1. Omura GA, Brady MF, Homesley HD, et al.: Long-term follow-up and prognostic factor analysis in advanced ovarian carcinoma: The Gynecologic Oncology Group experience. J Clin Oncol 1991; 9:1138–1150.
2. Silverberg SG: Prognostic significance of pathologic features of ovarian carcinoma. Current Topics in Pathology 1989;78:85–101.
3. Bell DA: Flow cytometry in ovarian neoplasms. Current Topics in Pathology 1992;85:337–356.
4. Vindelov LL, Christensen IJ, Nissen NI: A detergent-trypsin method of the preparation of nuclei for flow cytometric DNA analysis. Cytometry 1983;3:323–327.
5. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–481.
6. Rodenburg CJ, Cornelisse CH, Heintz PAM, et al.: Tumor ploidy as a major prognostic factor in advanced ovarian cancer. Cancer 1987;59:317–323.
7. Kallioniemi OP, Punnonen R, Mattila J, et al.: Prognostic significance of DNA index, multiploidy, and s-phase fraction in ovarian cancer. Cancer 1988;61:334–339.
8. Iversen OE: Prognostic value of flow cytometric DNA index in human ovarian carcinoma. Cancer 1988;61:971–975.
9. Friedlander ML, Hedley DW, Swanson C, Russell P: Prediction of long term survival by flow cytometric analysis of cellular DNA content in patients with advanced ovarian cancer. J Clin Oncol 1988;6:282–290.
10. Barnabei VM, Miller DS, Bauer KD, et al.: Flow cytometric evaluation of epithelial ovarian cancer. Am J Obstet Gynecol 1990;162:1584–1592.
11. Lage JM, Weinberg DS, Huettner PC, Mark SD: Flow cytometric analysis of nuclear DNA content in ovarian tumors: Association of

ploidy with tumor type, histologic grade, and clinical stage. Cancer 1992;69:2668–2675.
12. Pfisterer J, Kommoss F, Sauerbrei W, et al.: Cellular DNA content and survival in advanced ovarian carcinoma. Cancer 1994;74: 2509–2515.
13. Gajewski WH, Fuller AF, Pastel-Ley C, et al.: Prognostic significance of DNA content in epithelial ovarian cancer. Gynecol Oncol 1994;53:5–12.
14. Zanetta G, Keeney G, Cha S, et al.: The prognostic significance on long-term survival of flow cytometric DNA measurement in advanced ovarian cancer (OC) [abstract]. Proc Am Soc Clin Oncol 1995;14:274.
15. Rice LW, Mark SD, Berkowitz RS, et al.: Clinicopathologic variables, operative characteristics, and DNA ploidy in predicting outcome in ovarian epithelial carcinoma. Obstet Gynecol 1995; 86:379–385.